LIPID-NUCLEIC ACID PARTICLES PREPARED VIA A HYDROPHOBIC LIPID-NUCLEIC ACID COMPLEX INTERMEDIATE AND USE FOR GENE TRANSFER

FIELD OF THE INVENTION

This invention relates to methods of preparing lipid-nucleic acid particles which are useful for the introduction of nucleic acids into cells. The lipid-nucleic acid particles prepared by this method are stable in vivo and are suitable as nucleic acid or antisense transfer delivery vehicles, practical for clinical use.

BACKGROUND OF THE INVENTION

Developments in recombinant deoxyribonucleic acid ("DNA") technology have opened up new avenues for medical treatment. The location and sequences of an increasing number of disease-related genes are being identified, and clinical testing of nucleic acid-based therapeutics for a variety of diseases is now underway.

Gene therapy involves the introduction of genetic material into a cell to facilitate expression of a deficient or defective protein. Missing or defective genes (sequences of DNA encoding messenger RNA which are used as templates for protein construction) which are responsible for the production of these proteins result in a class of genetic disease often referred to as 'inborn errors of metabolism'. In some cases the disease can be treated by controlling the diet, as in the case of phenylketonuria, in which the liver enzyme responsible for the conversion of phenylalanine to tyrosine is defective. Untreated, this disease can result in mental retardation.

Treatments available for most genetic diseases are not as straightforward as merely altering the diet. For example, adenosine deaminase (ADA) deficiency results from a missing or defective gene that makes the adenosine deaminase enzyme. This enzyme is essential for a healthy immune system. ADA deficiency, however, is the disease successfully treated by the first human "gene transfer" experiment conducted by Kenneth Culver in 1990 (see, Culver, GENE THERAPY: A HANDBOOK FOR PHYSICIANS, Mary-Ann Liebert, Inc. publishers, p. 33–40 (1994)).

One method of introducing nucleic acids into a cell is mechanically, using direct microinjection. However this method is only practical for transfecting eukaryotic germline cells for the production of transgenic systems. To be effective in treating a disease, a nucleic acid-based therapy must enter many cells.

Systemic gene transfer entails distributing nucleic acids to target cells and then transferring the nucleic acid across a target cell membrane intact and in a form that can function in a therapeutic manner. In vivo gene transfer is complicated by serum interactions, immune clearance, toxicity and biodistribution.

The in vivo gene transfer methods under study in the clinic consist almost entirely of vital vectors. Although vital vectors have the inherent ability to transport nucleic acids across cell membranes and some can integrate exogenous DNA into the chromosomes, they can carry only limited amounts of DNA and also pose risks. One such risk involves the random integration of viral genetic sequences into patient chromosomes, potentially damaging the genome and possibly inducing a malignant transformation. Another risk is that the vital vector may revert to a pathogenic genotype either through mutation or genetic exchange with a wild type virus.

Lipid-based vectors have also been used in gene transfer and have been formulated in one of two ways. In one method, the nucleic acid is introduced into preformed liposomes made of mixture of cationic lipids and neutral lipids. The complexes thus formed have undefined and complicated structures and the transfection efficiency is severely reduced by the presence of serum. Preformed liposomes are commercially available as LIPOFECTIN® and LIPOFECTAMINE®. The second method involves the formation of DNA complexes with mono- or poly-cationic lipids without the presence of a neutral lipid. These complexes are prepared in the presence of ethanol and are not stable in water. Additionally, these complexes are adversely affected by serum (see, Behr, *Acc. Chem. Res.* 26:274–78 (1993)). An example of a commercially available poly-cationic lipid is TRANSFECTAM®.

Other efforts to encapsulate DNA in lipid-based formulations have not overcome these problems (see, Szoka, et al., *Ann. Rev. Biophys. Bioeng.* 9:467 (1980); and Deamer, U.S. Pat. No. 4,515,736).

Ideally, a delivery vehicle for nucleic acid will be small enough (<200 nm) and stable enough in circulation to distribute from local injection sites or following intravenous injection. The composition will have the maximum amount of nucleic acid per particle and will be homogenous and reproducible. The composition should also maintain the nucleic acid in a configuration which is protected from degradation prior to nuclear delivery and should efficiently transfect the target cells.

Surprisingly, the present invention provides such compositions and methods for their preparation.

SUMMARY OF THE INVENTION

The present invention provides novel, lipid-nucleic acid particles via formation of hydrophobic lipid-nucleic acid complexes. The complexes are charge-neutralized. Formation of these complexes in either detergent-based or organic solvent-based systems, followed by removal of the detergent or organic solvent, leads to particle formation.

Thus, the present invention also provides methods of preparing lipid-nucleic acid particles which are useful for the therapeutic delivery of nucleic acids. The particles are constructed via a hydrophobic lipid-nucleic acid intermediate (or complex). Upon removal of a solubilizing component (i.e., detergent or an organic solvent) the nucleic acid becomes protected from degradation. The particles thus formed are suitable for use in intravenous nucleic acid transfer as they are stable in circulation, of a size required for pharmacodynamic behavior resulting in access to extravascular sites and target cell populations.

Briefly, one method of forming lipid-nucleic acid particles, involves:

(a) contacting nucleic acids with a solution of non-cationic lipids and a detergent to form a nucleic acid-lipid mixture;

(b) contacting cationic lipids with the nucleic acid-lipid mixture to neutralize the negative charge of said nucleic acids and form a charge-neutralized mixture of nucleic acids and lipids: and

(c) removing the detergent from the charge-neutralized mixture to provide the lipid-nucleic acid particles in which the nucleic acids are protected from degradation.

Another method of forming lipid-nucleic add particles, involves:

(a) contacting an amount of cationic lipids with nucleic acids in a solution; the solution comprising of from